Mitogen dose-dependent effect of weak pulsed electromagnetic field on lymphocyte blastogenesis

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Received 10 February 1986

The effects of pulsed extremely low frequency electromagnetic fields on human peripheral blood lymphocyte mitogenesis induced by phytohaemoagglutinin, concanavalin A or calcium ionophore A23187 were studied. The dependence of the field effect on mitogen concentrations was investigated. Field exposure produced strong inhibition of DNA synthesis when optimal doses of mitogens were used, confirming our previous findings. Opposite effects were observed at suboptimal concentration of mitogens. Experiments performed by exposing cell cultures to the field for short periods indicated that a field application of at least 6 h is needed to influence irreversibly lymphocyte blastogenesis.

Electromagnetic field *Lymphocyte* *Mitogenesis* *Lectin* *Ionophore A23187*

1. INTRODUCTION

There is increasing evidence suggesting that electromagnetic fields (EMF) at extremely low frequency (ELF) may influence several cell functions [1,2].

Recently, Blackman et al. [3] have demonstrated increased calcium efflux from chick brain by combining low-frequency sinusoidal EMF and static magnetic fields of the order of the local Earth's field. On the basis of this and similar results [4], Liboff [5] has proposed a mechanism which implies cyclotron resonance of ions stimulating their transport through membrane channels.

We have previously reported that a pulsed EMF markedly reduces DNA synthesis in human lymphocytes stimulated with non-specific mitogens [6]. Alteration of calcium fluxes can be involved in the observed effect. We have in fact previously shown reduced ^{45}Ca uptake by stimulated lymphocytes after 1 h EMF exposure [7].

There is general agreement that at least two signals are required for proliferation of resting lymphocytes [8,9]. Calcium influx into the cell mediated by an ionophore or induced by binding of mitogenic lectins on the cell membrane plays an important part in the triggering of lymphocyte proliferation [10–13]. Thus, the mitogenic response of lymphocytes is dependent on extracellular calcium [14–16] although the precise nature of this dependence is controversial [17–19].

It is known that mitogen stimulation is a dose-related phenomenon for which there is an optimum concentration. Moreover, there is a good correlation between mitogen concentration and calcium influx-efflux [20–23].

Here, we have investigated the dependence of field effect on mitogen concentration. In addition to concanavalin A (Con A) and phytohaemagglutinin (PHA), we have also studied the effect of EMF on the action of the cation ionophore A23187. We further examined the reversibility of the action of EMF by exposing cells to the field for short periods only.

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/86/$3.50

2. MATERIALS AND METHODS

2.1. *Cells*

Human peripheral blood lymphocytes (HPBL) were isolated from heparinized venous blood from healthy volunteers on Ficoll-Hypaque (Pharmacia) density gradients. The cells were washed 3 times and resuspended at the desired concentration in RPMI 1640 (Gibco) containing 10% fetal bovine serum, 2 mM glutamine, penicillin (25 IU/ml), streptomycin (0.25 mg/ml). 100-μl aliquots of the HPBL suspension (2×10^5 cells) were established in 96-well microtiter plates (Falcon). PHA-P (Difco) (100 μl) or Con A (Calbiochem) (100 μl) were added to the cultures at final concentrations ranging from 0.2 to 2×10^3 μg/ml and from 0.1 to 100 μg/ml, respectively. Stock solution of ionophore A23187 (Sigma) was prepared in dimethyl sulfoxide (DMSO) at 10 mg/ml. Dilutions of ionophore were made directly in RPMI 1640. Final concentrations of A23187 used were 0.5, 1 and 2 μM. The effects of DMSO alone at the concentration used were evaluated. No significant difference in [^{3}H]thymidine incorporation was observed in comparison with controls. Cell cultures were incubated in 96-well plastic microtiter plates (diameter 6.5 mm/well) for 72 h at 37°C in a 5% CO_2 atmosphere and pulsed with [^{3}H]thymidine, 2 μCi/ml (Amersham, spec. act. 25 Ci/mmol), for the last 6 h of culture. The cells were then harvested on an automated multiple-sample harvester and the cell-associated radioactivity counted in a Beckman liquid scintillation counter.

2.2. *Exposure system*

The EMF was generated by passing a current through a pair of concentric 966-turn coils, 10 cm in radius, separated by 2 cm. This device was wired in parallel to a pulse generator which gave a train of square pulses of frequency 3 Hz. The generated field had an intensity of ~50 G and 30 ms rise time. The wave form of the current passing through the coils was only weakly smoothed due to the inductance of the coil system which was 249 mH. The magnetic field was checked by using a Hall-effect probe. The coils were placed in a tissue incubator held at 37°C, while the pulse generator was outside. The temperature between the coils was checked with a digital thermometer and remained constant at 37°C. The cultures to be exposed to EMF were placed in the central space between the coils. Only those wells showing an EMF homogeneity better than 1% were used.

2.3. *Statistical analysis*

Significance of the results was analysed by the two-way analysis of variance as highly significant differences between experiments in the response levels were observed, a known problem in mitogenic assays [24]. The Fisher variance ratios were calculated to evaluate the significance of both results between experiments and field effectiveness.

3. RESULTS

The effect of a 3 Hz EMF on the PHA-induced proliferative response of HPBL is shown in fig.1. It is evident that [^{3}H]thymidine incorporation was significantly lower after EMF exposure when an optimal (20 μg/ml) or higher dose (0.2 and 2 mg/ml) of mitogen was used. EMF did not show any significant effect at a PHA concentration of 2 μg/ml while at 0.2 μg/ml, [^{3}H]thymidine incor-

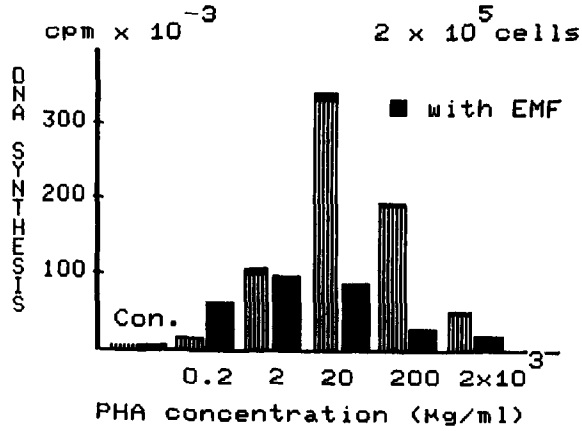

Fig.1. Effect of a 3 Hz square wave form EMF on PHA-induced DNA synthesis in human peripheral blood lymphocytes. [^{3}H]Thymidine incorporation into DNA is shown for 5 PHA concentrations. Data represent means ± SE of triplicate determinations in each of 6 experiments. Differences between exposed and unexposed cultures are highly significant ($P < 0.0001$) at 0.2 μg/ml ($F = 179$), 20 μg/ml ($F = 336$), 200 μg/ml ($F = 439$) and 2 mg/ml ($F = 56.2$); not significant for controls (con) ($F = 0.25$) and at 2 μg/ml ($F = 0.002$).

poration in lymphocytes was significantly higher than that obtained without the field. Similar results were obtained using Con A as the mitogen (fig.2). Also in this case, EMF exposure resulted in markedly lower [^{3}H]thymidine incorporation than that measured with the field off at optimal Con A concentration (5 μg/ml). At suboptimal doses a stimulating effect of the field was observed. At 10 μg/ml Con A the field action was not significantly effective. As can be appreciated from fig.3, EMF was also capable of significantly affecting DNA synthesis in lymphocytes treated with ionophore A23187.

It is evident that a pronounced stimulant effect of the field occurred when a suboptimal concentration of A23187 (0.5 μM) was used while at optimal A23187 concentration (1 μM) there was decreased [^{3}H]thymidine incorporation after EMF application. At 2 μM ionophore the field had no significant effect on lymphocyte DNA synthesis.

3.1. *Shorter exposure to EMF*

In another set of experiments, the lymphocyte cultures were exposed to a 3 Hz EMF during the first hour of incubation only. After this time the cultures were carried outside the field for 71 h. The

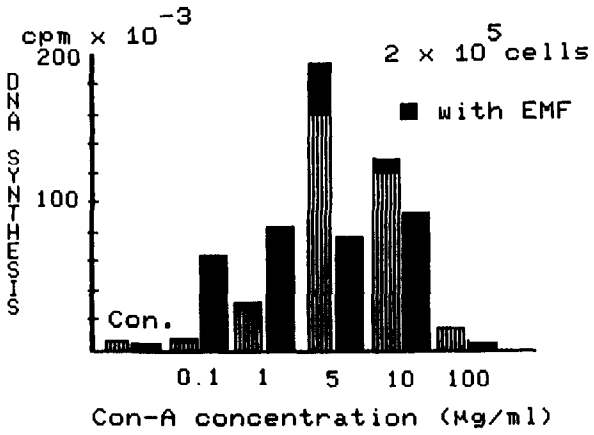

Fig.2. Effect of a 3 Hz square wave form EMF on Con A-induced DNA synthesis in human peripheral blood lymphocytes. [^{3}H]Thymidine incorporation into DNA is shown for 5 Con A concentrations. Data represent means ± SE of triplicate determinations in each of 5 experiments. The differences between exposed and unexposed cultures are highly significant ($P < 0.0001$) at 0.1 μg/ml ($F = 242$), 1 μg/ml ($F = 85.9$), 5 μg/ml ($F = 59$), 100 μg/ml ($F = 42$); not significant for controls (con) ($F = 0.25$) and at 10 μg/ml ($F = 4.2$).

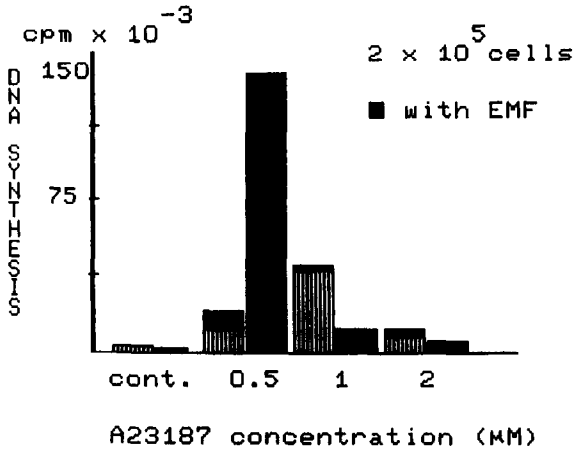

Fig.3. Effect of a 3 Hz square wave form EMF on A23187-induced DNA synthesis in human peripheral blood lymphocytes. [^{3}H]Thymidine incorporation into DNA is shown for 3 ionophore concentrations. Data represent means ± SE of 6 determinations in each of 4 experiments. Differences between exposed and unexposed cultures are highly significant ($P < 0.0001$) at 0.5 μM ($F = 161.6$), 1 μM ($F = 67.7$); not significant for controls ($F = 1.2$) and at 2 μM ($F = 0.33$).

results obtained (not shown) indicate that the application of EMF limited to this early time was not sufficient to induce a permanent effect on lymphocytes treated with PHA or Con A. Instead, when the cells were exposed to the field for the first 6 h of incubation, PHA or Con A stimulation produced significant effects, as shown in fig.4.

4. DISCUSSION

We have previously studied the influence of a 3 Hz square wave form EMF on lymphocytes stimulated by lectins, observing an inhibitory effect on DNA synthesis for optimal concentrations of Con A and PHA [6,7]. These findings appear to be largely confirmed by the present results. Nevertheless, the results also show a modulatory effect of EMF on lymphocyte blastogenesis, stimulatory at suboptimal lectin concentrations and inhibitory otherwise.

The possibility of an interaction of EMF with the biological events involved in primary lymphocyte stimulation (e.g. formation of lectin-receptor complexes, movements of Ca^{2+}, etc.) is generally accepted, even if many authors have

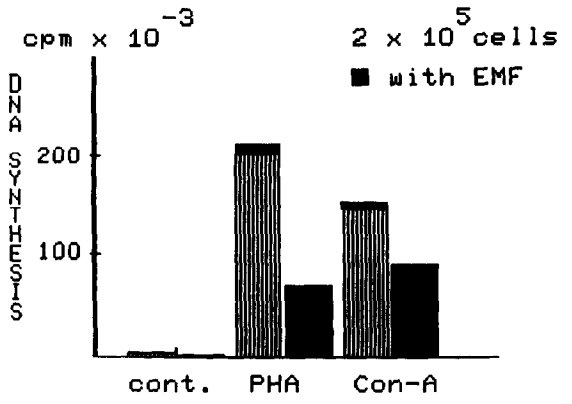

Fig.4. [^{3}H]Thymidine incorporation in human peripheral blood lymphocytes unstimulated or stimulated with optimal concentrations of PHA (20 μg/ml) and Con A (5 μg/ml) and exposed to a 3 Hz square wave form EMF for the first 6 h of incubation. Data represent means ± SE of 6 determinations in each of 3 experiments. Differences between exposed and unexposed cultures are highly significant ($P < 0.0001$) for PHA-stimulated cells ($F = 71$) and Con A-stimulated cells ($F = 21.6$); not significant for controls ($F = 0.016$).

pointed out that the conceivable energy transfer from the EMF to the biological systems is too small to affect its behaviour in the absence of an amplification process.

More recently, Liboff [5] proposed that the cyclotron resonance phenomenon may account for the results of Blackman et al. [3] showing enhanced Ca^{2+} efflux from chick brain with particular combinations of a weak static magnetic field and a sinusoidal EMF. The basic idea is that the energy transfer occurs when ions are located in the ionic channels which have an elicoidal internal structure. This suggestive hypothesis is supported by the theoretical consideration that, for a magnetic field of the order of the Earth's magnetic field, all resonance frequencies for ions of biological interest fall within the ELF region. Therefore, the ELF EMF could be able to force the ions through the channels present in the membranes. Moreover, much experimental evidence of biological effects in the ELF region has been reported for pulsed EMF containing many low- and high-frequency components [1,6]. In particular, the square wave form used here contains all low-frequency multiples of the repetition frequency (e.g. 3 Hz) and also many high-frequency components with lower intensity. This large spectrum of frequencies could also create, in the presence of a weak static magnetic field (e.g. drift current components), the conditions of resonance for many ionic species in their hydrated form. The concomitant action of ELF and lectins or ionophore seems necessary to detect the action of the field on the mitogenesis of cell cultures, as the field alone was not effective. The presence of the lectins or ionophore may be needed for bringing ions inside the membrane where an amplification mechanism allows the EMF to facilitate the ionic flux across the membrane. The last hypothesis could find experimental evidence in the present results of a striking stimulatory effect of the EMF at suboptimal concentrations of lectins and ionophore. In the latter case the effect is very strong, as expected for a channel-forming substance.

Our previous finding of a reduced Ca^{2+} influx in lymphocytes exposed for 1 h to EMF and stimulated with optimal concentrations of mitogen [7] does not disagree with the present results obtained at various mitogen doses. There is in fact a close correlation between calcium fluxes and mitogen concentration [22,23]. EMF could induce facilitated calcium influx which is smaller than the optimal value but still sufficient to produce stimulation. From this point of view, the EMF effect on induced lymphocytes appears independent of mitogen concentration. Some authors [21] reported that at low concentrations of Con A no increase in influx took place while enhanced Ca^{2+} efflux occurred. Recently, using PHA and A23187 on human lymphocytes, it was concluded that although substantial increase in total cellular Ca^{2+} may not be essential for mitogenesis, an increase in $^{45}Ca^{2+}$ exchange is closely associated with the mitogenic effect of PHA and A23187 [20]. Thus, it is also possible that EMF induces increased calcium exchange between the external medium and the cell leading to cell proliferation. A further possibility is a different intracellular distribution of calcium induced by the combined action of EMF and mitogen.

The results of experiments performed by exposing the cells to EMF for short periods indicate that application for 6 h is needed to induce an irreversible effect on lymphocyte mitogenesis.

Experimental proof of the proposed physical

mechanism of interaction between EMF and cellular processes has not yet been provided. In further studies it will be necessary to change the exposure conditions and to measure carefully the ionic exchanges.

ACKNOWLEDGEMENT

We wish to thank Professor A. Finazzi-Agrò for his critical review of the manuscript.

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